Radiation Effects on the Metabolism of Phospholipids

in the Central Nervous System of Albino Rats

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Mechanism of Action of X-Rays on Phospholipids and Precursors

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Submitted by

Charlotte O. Lee, Ph.D.
Professor of Chemistry
Principal Investigator

Alabama Agricultural and Mechanical College
Normal, Alabama

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Charlotte O. Lee
Charlotte O. Lee, Principal Investigator

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Abstract

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Studies on the isolation and characterization of breakdown products formed as a result of x-irradiation of phospholipids and precursors are presented in this report. Chromatogram plates containing the compounds, ceramide, lysolecithin, phosphatidylserine, ethanolamine, choline chloride, and other phospholipids were irradiated directly; and chromatographic separation was completed in from five to twenty-six hours post radiation. G-values were calculated in some instances.

Mechanism of Action of X-Rays on Phospholipids and Precursors

Introduction

As set forth in the proposal "Radiation Effects on the Metabolism of Phospholipids in the Central Nervous System of Albino Rats," three levels of organization were to be included in long term studies relative to the effects of radiation on phospholipid metabolism, (1) the molecular level, (2) the subcellular level, and (3) the organism level. Studies on the molecular level were to include the following:

a. The effects of x-rays on chemical structure of phospholipids and phospholipid precursors.

b. The effects of x-rays on the chemical and biochemical synthesis of phospholipids, especially sphingomyelin.

c. The isolation, purification, and characterization (with respects to radiation effects) of the enzyme, phosphorylcholine-ceramide transferase and phosphorylcholine-glyceride transferase.

The molecular level of organization has been investigated, and only certain aspects of (a) and (b) have been studied to any great extent. Experiments involving item (c) are now in progress.

Spectroscopic and chromatographic evidence for the alteration of the molecular structure of phospholipids and related compounds was reported in Progress Reports I (July 1, 1965-January 31, 1966) and Progress Report II (February 1, 1966 to August 31, 1966).

Analyses of infrared and ultraviolet spectra showed the intensification of carbonyl and amino group frequencies in x-irradiated phospholipids. At least two components were present in thin-layer chromatograms of N-Acylsphingosine (ceramide), choline chloride, lecithin, sphingomyelin and cephalin. In the absence of oxygen and aqueous solvents, structural changes were apparent in irradiated compounds. Powers and co-workers (1961) described oxygen-independent, temperature dependent irradiation induced changes as Class I type damage. Pasynskii and co-workers (1964) believed that Class I damage was due to direct radiation effects on a very small number of molecules. From our observations, this damage must follow a somewhat random course.

A kinetic model for radiation damage based upon the work done by Lindblom (1961) with choline chloride was proposed for ceramide in Progress Report III (September 1, 1966 to December 31, 1966).

The purpose of this report is to present the findings of studies designed to test the model proposed in Progress Report III.

Procedures

Because radiation damage can occur even in the absence of oxygen, attempts were made to exclude oxygen only when solutions were irradiated. To facilitate the isolation and study of irradiation produced intermediates, thin-layer chromatogram plates were loaded with 200-500 micrograms of the compounds to be tested; and the whole chromatogram, along with sufficient blank plates, was irradiated at room temperature. All samples were spotted on Eastman TLC plates, Gelman I TLC plates, types SG and A. One experimental and one standard plate of each type of chromatogram sheet was prepared for all samples studied. The compounds so treated were phosphatidylserine (Mann Research Laboratories), sphingomyelin (Calbiochem, bovine brain [B Grade]), sphingosine (Miles Laboratories, DL-Erythroform), N-Acyl-sphingosine (Applied Sciences Laboratories, mixed ceramide), lysolecithin (Mann Research Laboratories, crystalline), cytidine diphosphate choline (Boehringer Mannheim Corporation), lecithin (Mann Research Laboratories), choline chloride, 2-aminoethanol (redistilled), 2-amino-2-methyl-propanol (Matheson, Coleman, and Bell), 2-methylaminoethanol (Eastman Organic Chemicals, redistilled), phosphatidylserine (Mann Research Laboratories) and DL-serine.

Irradiations were obtained from a Norelco MG 300 X-ray machine at a focal distance of eight inches. The total dosage ranged from 50,000 to 10,000r (300kv, 10ma), and the time of irradiation varied from 50 minutes to 10 minutes 8 seconds, respectively. Chromatograms were developed in solvents between five and twenty-six hours post radiation. Solvents used were chloroform; chloroform-methanol-water (90:10:1); chloroform-methanol-acetic acid-water (25:15:4:2, Parker, 1965); phenol-butanol (1:1) in an acetic acid atmosphere. Components of the irradiated compounds which gave positive reactions with 2,4-dinitrophenylhydrazine were scraped from the chromatograms, eluted with chloroform (using a fritted glass chromatography column), and the eluate read at 395mu in a Beckman Spectrophotometer by a modification of the procedures of Wittenberg, Korey and Swenson (1956) and Dhont and DeRooy (1961).

Results

Irradiation of thin-layer chromatographic plates were useful for the isolation of components formed by way of x-irradiation. Carbonyl compounds were visualized directly on the chromatogram plates, sprayed with 2,4-dinitrophenylhydrazine for choline chloride, aminoethanol, ceramide (N-Acylsphingosine), sphingomyelin, and phosphatidylserine. Qualitative tests are in progress to ascertain the identity of these carbonyl substances. Comparative studies with myristaldehyde indicate that G values for ceramide were about 121 molecules/100ev with an absorbed dose of 10,000r of 300kv x-rays. These values are only tentative because the entire series of experiments are being repeated.

The use of infrared spectroscopy to determine the relative destructive action of 10,50,000r of 300 kv x-rays on the phospholipids, phosphatidylserine, and lysolecithin has been partly successful.

Figure 1 is a calibration curve for the carbonyl absorption of pure lysolecithin at 5.7-5.8 μ. This band is due to the carbonyl ester band of lecithins and cephalins (Schwarz and co-workers, 1957). Irradiation of lysolecithin caused a reduction in the intensity of this band to about 80% of the original strength.

The ultraviolet spectra of lysolecithin is presented in figure 2. Appearance of the carbonyl as aldehyde or ketone increased with an increase in dose rate. Curve 2 shows the decrease in broad carbonyl absorption which was observed in the quantitative infrared data. However, a five fold increase in dose rate increased the aldehydic carbonyl at 260 mμ. Further, a gradual increase in ultraviolet carbonyl absorption was observed when phosphatidylserine was exposed to approximately 200,000r of x-rays as illustrated in figure 3 (Curve A). Presumably the aluminum absorbed some radiation and afforded some protection against the rearrangement to aldehydes (Curve B). The infrared spectrum showed a strong carbonyl ester absorption prior to irradiation.

Quantitative studies on the intermediates formed when serine and ethanolamine were exposed to x-rays are incomplete. Figure 4 shows a strong carbonyl absorption in the infrared spectrum of irradiated ethanolamine (note arrow) which was not present in the non-irradiated control.

Although no carbonyl absorption was observed in the infrared spectrum of irradiated DL-serine, this function was pronounced in the ultraviolet spectrum (see figure 5, Curve A).

Studies are presently underway on the effects of x-rays on the biosynthesis of sphingomyelin in rat brain and spinal tissues.

Summary

1. Chromatographic procedures have been employed in irradiation, separation, and characterization of the phospholipids and precursors, phosphatidylserine, lysolecithin, ceramide, ethanolamine, and choline chloride.

2. Carbonyl absorbing substances were isolated by a thin-layer chromatographic technique. Work is continuing on the characterization of these compounds.

3. Studies have been initiated on the effect of x-rays on the biosynthesis of sphingomyelin.

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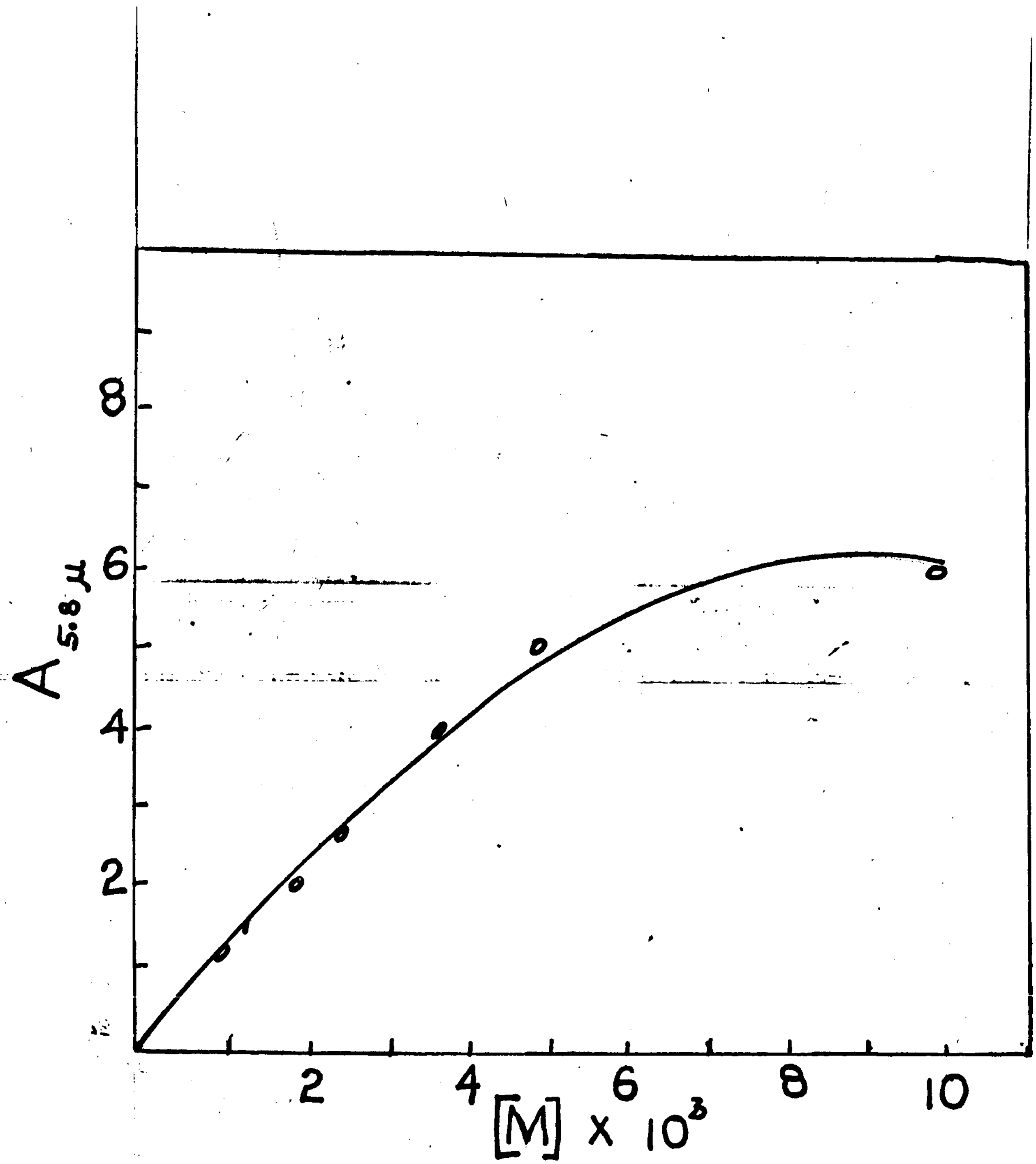

Figure 1. Infrared Calibration Curve for Lysolecithin Solvent CCl_4.

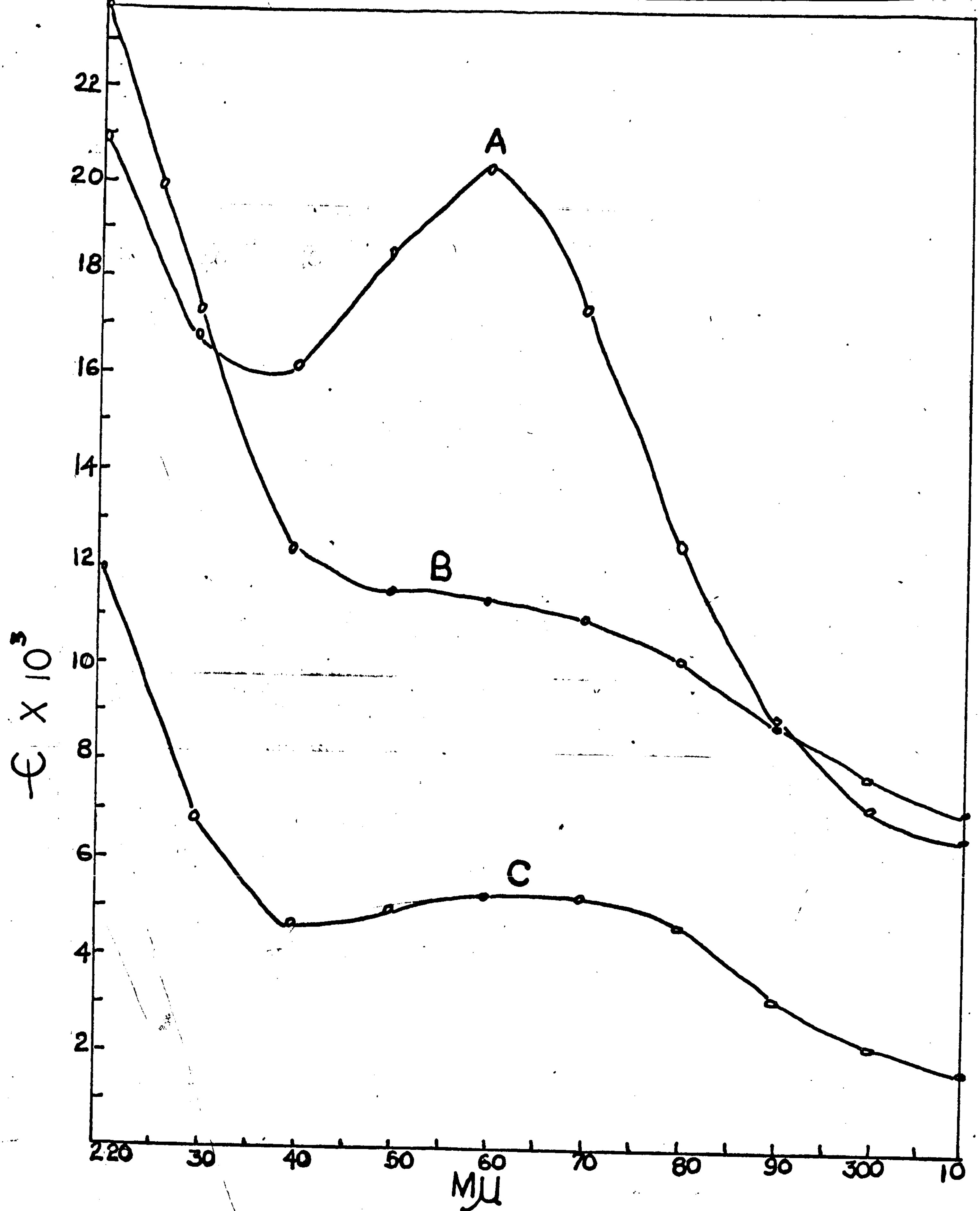

Figure 2. Appearance of Carbonyl Absorption Frequencies in the Ultraviolet Spectrum of Lysolecithin. Curve A is Lysolecithin IRRadiated (5×10^4r) in Carbontetrachloride; Curve B is control lysolecithin in the same solvent; Curve C is lysolecithin irradiated (1×10^4r) in carbontetra chloride.

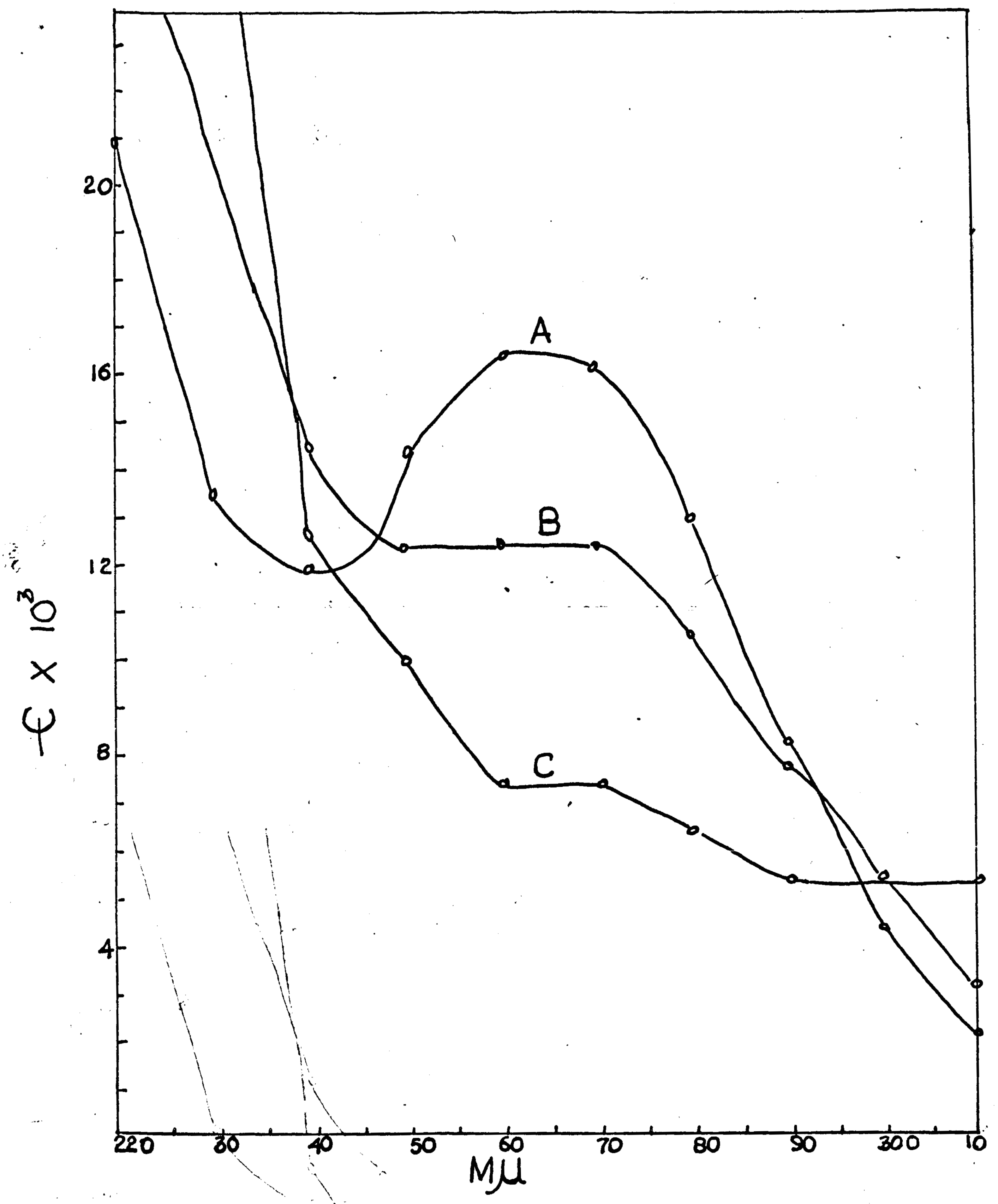

Figure 3. Appearance of Carbonyl Absorption Frequencies in Ultraviolet Spectrum of Phosphatidylserine. Curve A is phosphatidylserine (P-S) irradiated (1.97×10^5r) in CCl_4 in clear glass; Curve B is P-S irradiated (1.97×10^5r) in CCl_4 in vials wrapped in aluminum foil; Curve C is non-irradiated P-S.

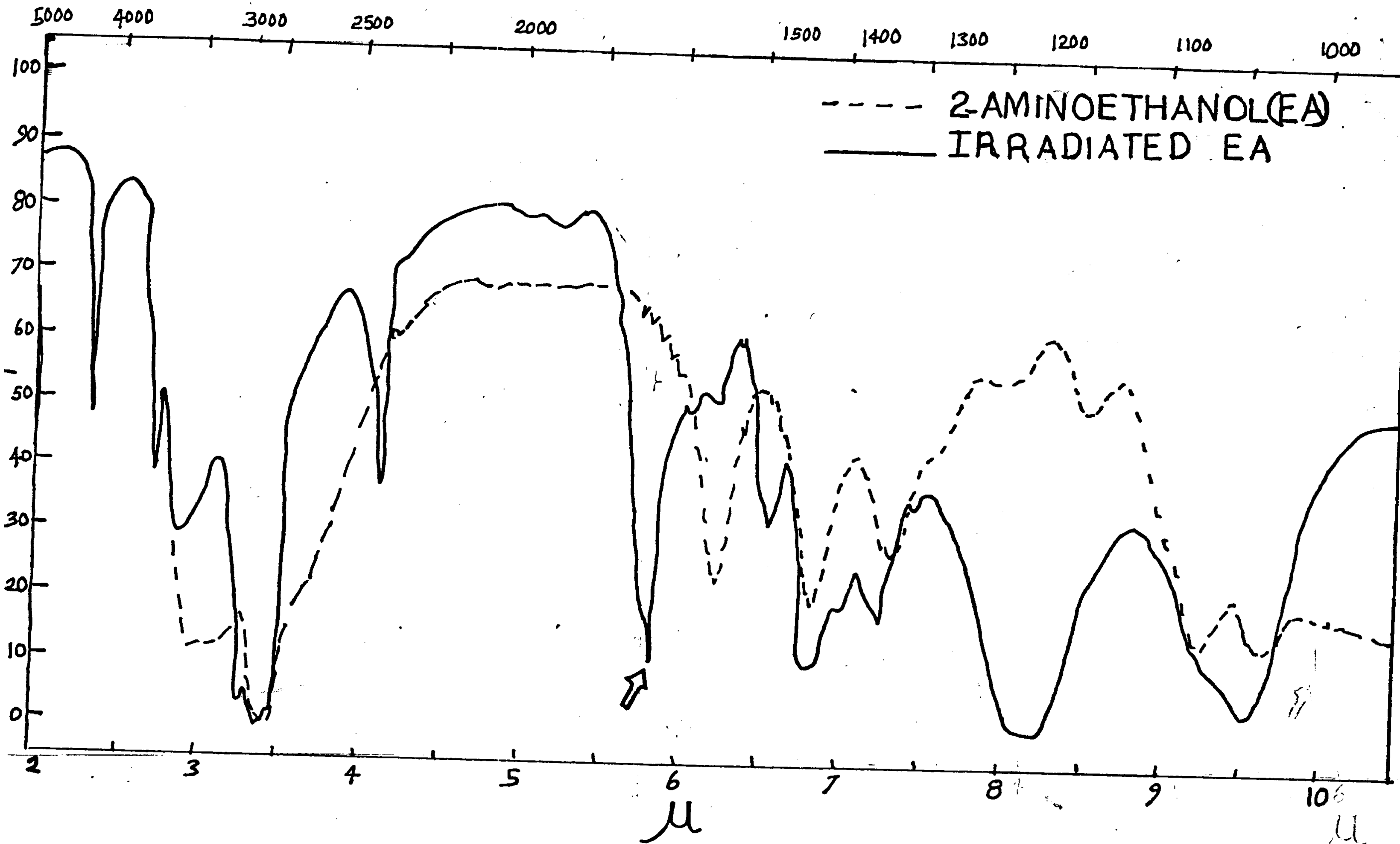

Figure 4. Infrared Spectra of Irradiated and Non-irradiated 2-Aminoethanol. A solvent was not used for IR Spectra nor irradiation.

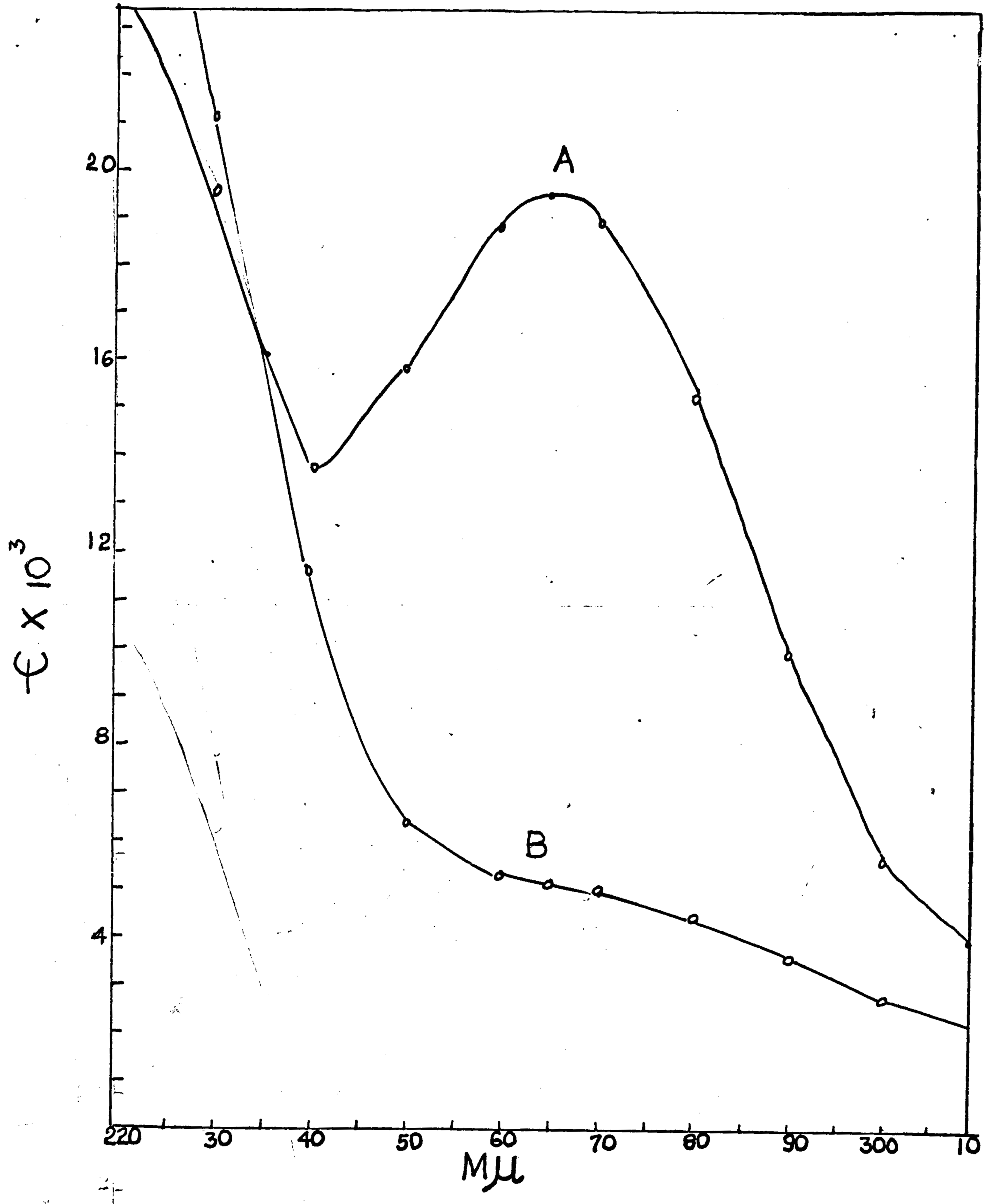

Figure 5. Appearance of Carbonyl Absorption Frequencies in Ultraviolet Spectrum of X-irradiated DL-Serine. Curve A was irradiated. Curve B is the non-irradiated control.

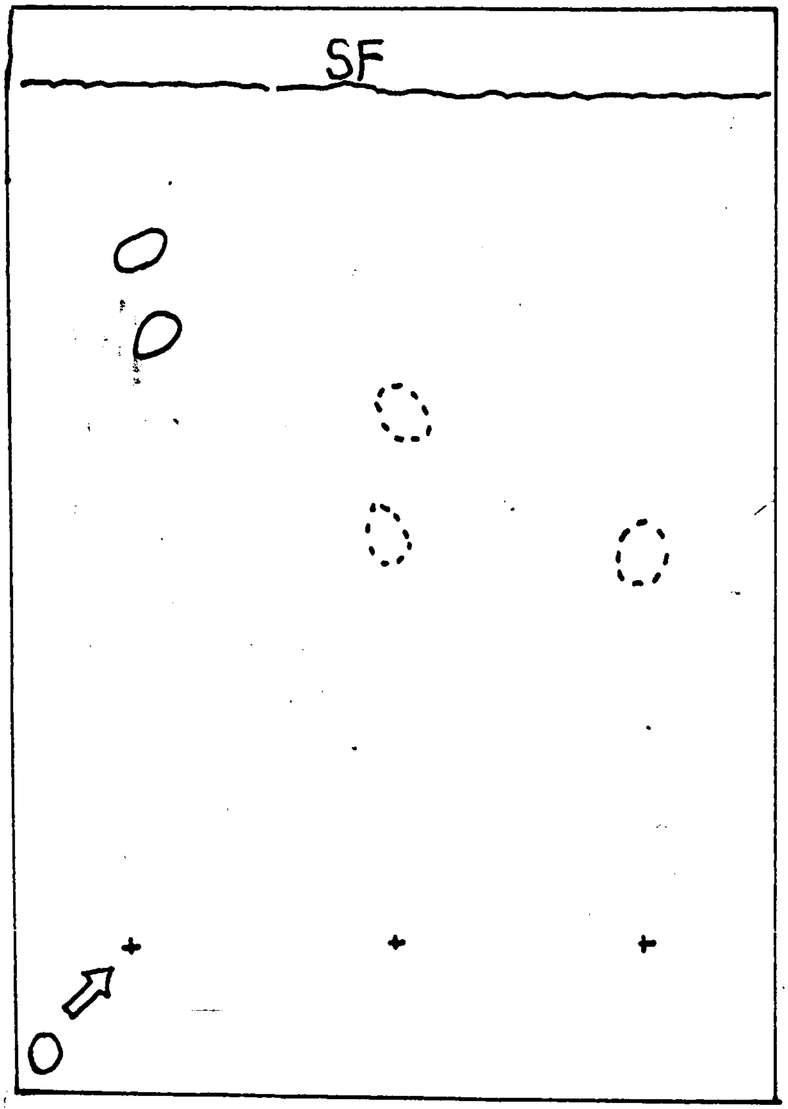

Figure 6-A. A Typical Thin-Layer Chromatography Chromatogram of Ceramide (C-Acyl-sphingosine). Components resulting from irradiation are represented by the dotted lines or circles. Solvent for chromatography was $CHCl_3$/MeOH/H_2O(90:10:1). Dose was 1 x 10^5r. O represents the origin and SF represents the solvent front.

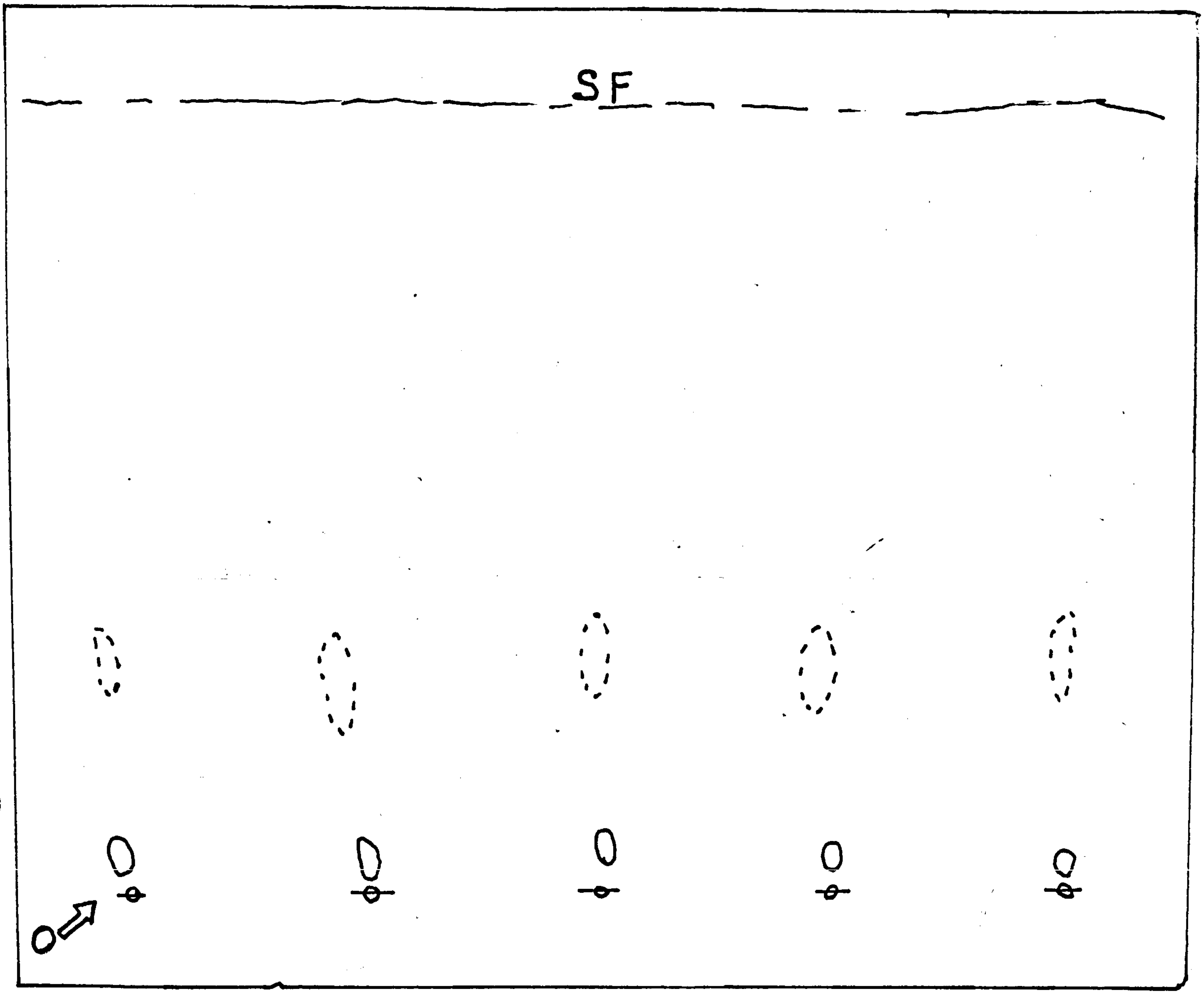

Figure 6-B. A Typical Thin-Layer Chromatography Chromatogram of Lysolecithin. Irradiation (dotted lines) was performed directly on chromatogram. Dose rate was 5 x 10^4r. Components were scraped off TLC plate, extracted with $CHCl_3$, pooled and read in UV for carbonyl absorption. O represents the origin and SF represents solvent front. Solvent same as in figure 6-A.

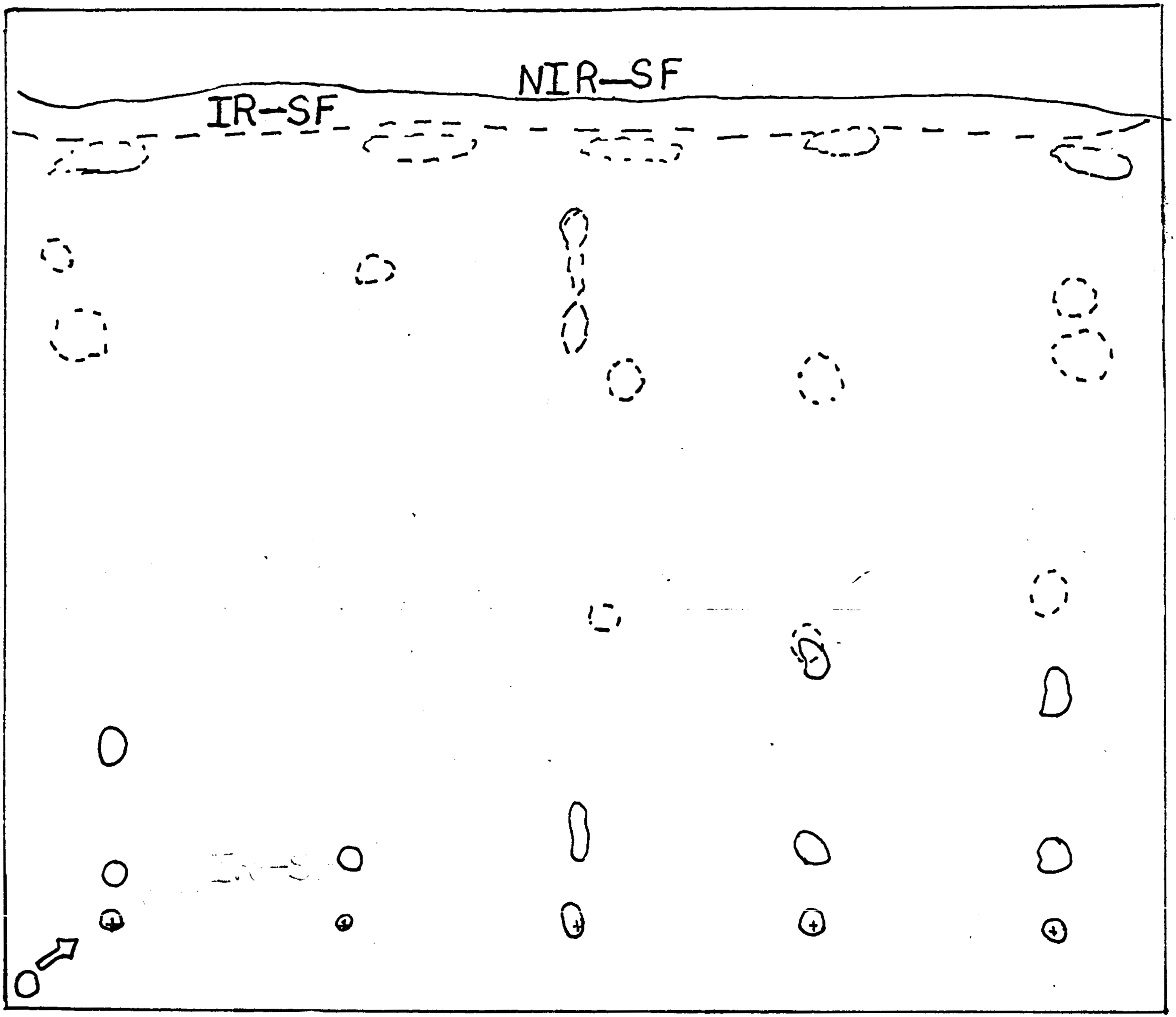

Figure 6-C. A Typical Thin-Layer Chromatography Chromatogram of Sphingomyelin. Irradiated (dotted lines) was performed directly on chromatogram. The dose was 5 x 10^4r of 300kv x-rays. Solvent same as for figure 6-A. NIR-SF = non-irradiated solvent front, IR-SF = irradiated solvent front. O = origin. Non-irradiated sphingomyelin was not chromatographically pure as indicated by two-three components (solid lines).

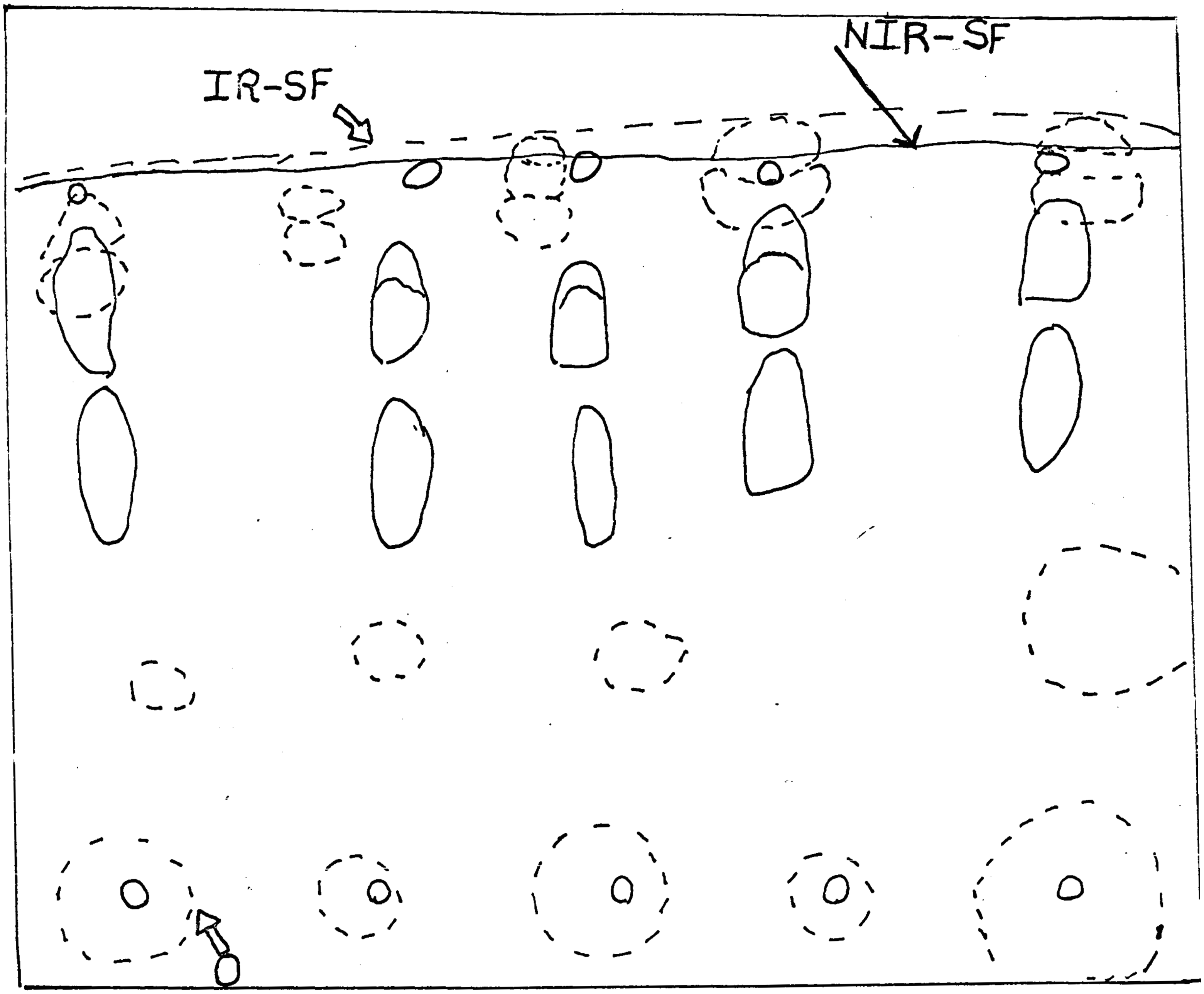

Figure 6-D. A Typical Thin-Layer Chromatography Chromatogram of Sphingosine. Solvent system same as in Figure 6-A. Irradiated chromatograms represented by dotted lines, non-irradiated chromatograms represented by solid lines. The irradiated components left at origin was ninhydrin positive. Note also that sphingosine was heterogeneous.